Comm.
Dr. Andervont
Dr. Gardner
Dr. Loosli
Dr. Sommers

CARCINOGENESIS

No. 603C

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date: July 6,1973

RECEIVED
JUL 11 1973

1. Principal Investigator (give title and degrees):

Shirley L. Kauffman, M.D.
Professor of Pathology

2. Institution & address:

State University of New York
Downstate Medical Center
450 Clarkson Avenue,
Brooklyn, N.Y. 11203

3. Department(s) where research will be done or collaboration provided:

Department of Pathology

4. Short title of study:

Pulmonary Alveolar Hyperplasia Induced by Carcinogens

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 2 years

7. Brief description of specific research aims: The general aim of the present study is to adapt morphometric methods, developed for analysis of growth of neonatal lung populations, to analysis of the preneoplastic growth phase induced by chemical carcinogens. The specific questions to be answered are:

1. Does alveolar epithelial hyperplasia occur following exposure to lung carcinogens other than urethane and if so, is the time sequence of the hyperplasia fixed?

2. Is there a relationship between aging and Type II cell hyperplasia which might underlie the finding that spontaneous tumors commonly occur in old mice.

100354042

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

8. Brief statement of working hypothesis: 2.

The hyperplastic growth phase of Type II alveolar cells demonstrated in mice continuously exposed to urethane in drinking water (1) may be a general phenomenon reflecting, in part the augmentation of "preneoplastic" cells. As such, it should be demonstrable following exposure of mice to injections of a variety of lung carcinogens. This may be a suitable model for study of early changes in carcinogens, as well as a possible biologic essay method for pulmonary carcinogen testing.

9. Details of experimental design and procedures (append extra pages as necessary)

100354043

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

Details of Experimental Design and Procedure

1. Relation of Hyperplasia to Neoplasia

Chimken and Pollisar (2) described hyperplasia of "lung cells" following a single injection of urethane. This preceded the development of lung tumors, and disappeared as tumors developed. Refinements of sectioning technique and use of morphometric autoradiographic method (Progress report Spring '73) have made it possible to identify the cells involved in the hyperplasia. These are the Type I and II alveolar cells, the latter the cell of origin of lung adenomas. The time sequence of this hyperplasia in mice continuously exposed to urethane in drinking water is shown in Figure 1 and 2. It has been suggested that hyperplasia may be dependent on the continuous presence of carcinogen (discussion macrophage Conference, The Council for Tobacco Research, N.Y. December 1972) and hyperplasia itself may amplify tumor yield by virtue of its associations which increased cell division. In order to control this factor, single injections of carcinogens will be used in the following experiments.

Experimental Plan

Adult mice will be exposed by single injection to 3 different pulmonary carcinogens, urethane, DMBA (9,10-Dimethyl-1,2 benzanthracene) and MNU (methyl nitrosourea) and the reaction of their pulmonary epithelium at intervals after injection will be analyzed by quantitative methods. Additional groups of injected and control animals will be killed at

100354044

Source: https://www.industrydocuments.ucsf.edu/docs/nzem0000

Details of Experimental Design and Procedure

10 months and the number of lung tumors counted. Table I summarizes the carcinogen, dose, weeks for sacrifice and incidence of tumors reported at 10 months along with references from which the data on dose and tumor yield were obtained.

TABLE FOR CARCINOGEN EXPOSURE IN ADULT MICE

Carcinogen	Dose/Body wt.	Sacrifice for Morphometry (Weeks)	Sacrifice for Tumors % Tumors	Reference
Urethane	1.0 mg/g	1,2,3,4,5,6	90% at 10 m	This laboratory
MNU	50.0 µg/g	1,2,3,4,5,6	100% at 10 m	(3)
DMBA	10.0 µg/g	1,2,3,4,5,6	100% at 10 m	(4)
Control	Saline	1,2,3,4,5,6	< 5% at 10 m	This laboratory

Method of Tissue Preparation

Mice will be anesthetized at stated intervals, a tracheostomy tube inserted for glutaraldehyde instillation and a bilateral pneumothorax performed immediately before instillation. After 2 hour fixation, the mediastinal contents will be dissected from the lung and lung volume measured by water displacement. Multiple 1 mm cubes of lung tissue will be processed for Epon embedding and subsequently cut at 1.5 micra, placed on glass slides and stained with Toluidene Blue.

Method of Tissue Analysis

Slides will be coded and examined in the Wild M-501 sampling microscope; identity and the numbers of cells in each microscopic field will be determined in 60-100 test areas from each animal's

100354045

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

Details of Experimental Design and Procedure

lung (3 animals/group). The number of Type I and II cells/cm^3 of lung can be calculated for each animal using the relation $Nv=Na/_D$ where Nv is the number of nuclei per unit volume, Na is the number of nuclei per unit section area, and D is the nuclear diameter. The number of Type I and II nuclei can then be calculated by multiplying the numerical density by the volume of lung parenchyma. (5)

The final groups (12 animals/groups) will be killed after 10 months and the number of superficial adenomas in the whole lung, and in serial section of one lobe will be determined.

These experiments should answer the following questions:

1. Is the pattern of alveolar epithelial hyperplasia which is found with continuous urethane exposure also found after a single urethane injection?

2. Do the carcinogens MNU and DMBA elicit a hyperplasic response?

3. If so, does the time sequence vary with the carcinogen and can it in any way be related to the final number of tumors produced?

1003544046

Source: https://www.industrydocuments.ucsf.edu/docs/nzcn0000

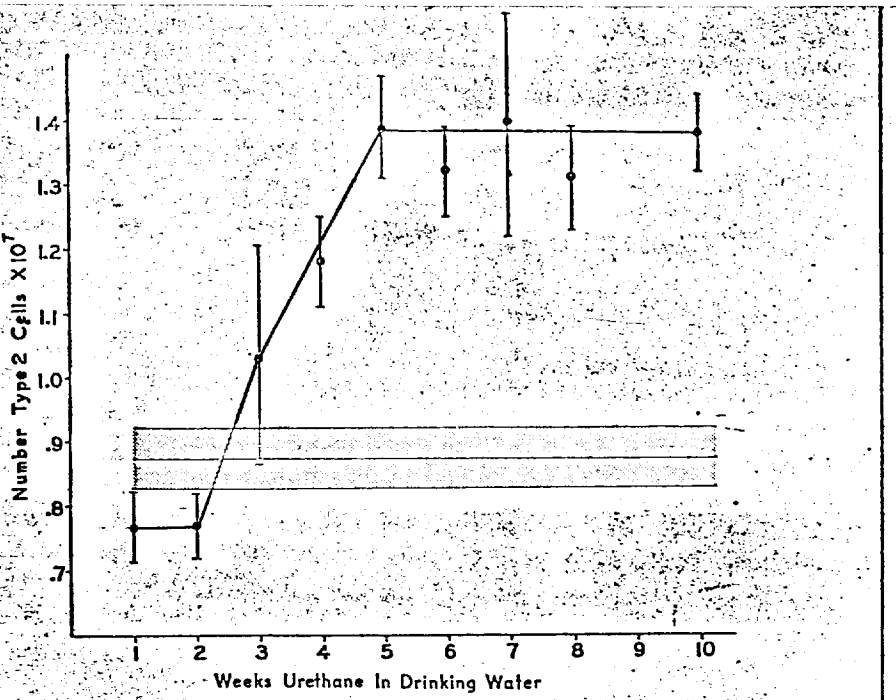


Figure 1

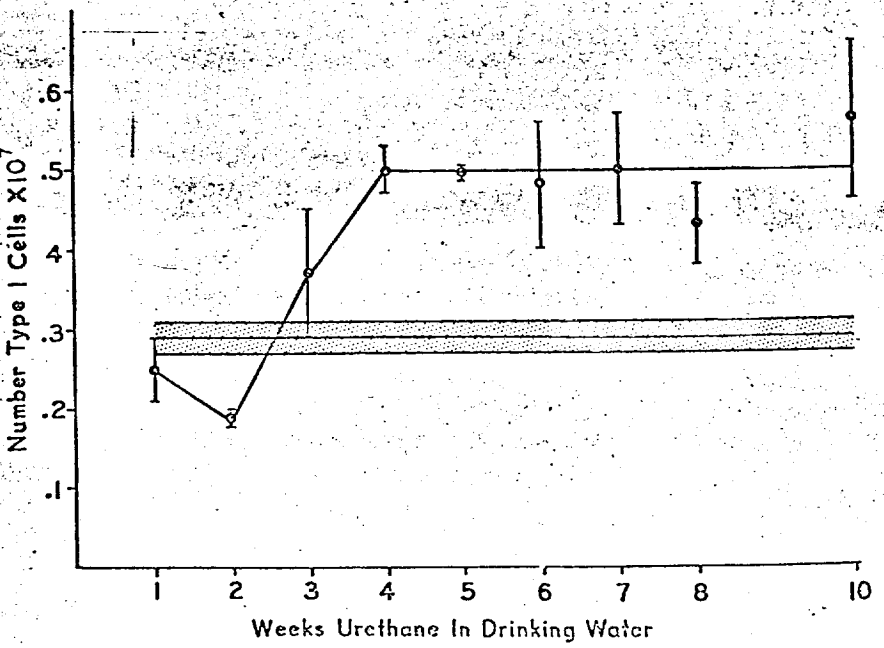


Figure 2

1003544047

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

Details of Experimental Design and Procedure

2. Life Cycle of epithelium in relation to Hyperplasia

Knowledge of Type II cell cycle and the growing evidence that Type II cells are probably the stem cell of alveolar epithelium (6, 7, 8) introduces the question of maturation and differentiation of this system. If the Type I cell arises from Type II stem cell, and a steady state is maintained, then some mode of exit of Type I cells from the population would be expected.

Alternately, Type I cells may not constitute a steady-state system and may have no turnover. In the latter case Type I cell population would increase throughout the life of the individual. No previous study has investigated alveolar epithelium as an expanding population. The question as to whether increase in cell numbers or hyperplasia may be a natural component of aging could be answered by a study of population kinetics.

Two types of experiments will be done:

A) Aging Experiment

In this long term experiment, lungs of mice from 6 months to 24 months of age will be studied using autoradiographic and morphometric methods to quantitate cell population numbers and obtain labeling indices.

Swiss Webster mice will be maintained under normal conditions for a maximum period of 16 months, with groups sacrificed at 4 month intervals, beginning at 6 m of age. ^{3}H thymidine (1 μc/gram:3.0 c/mM) will be injected into mice 60 minutes prior to sacrifice and tissue will be fixed and processed for autoradiography and morphometry

100354048

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

Details of Experimental Design and Procedure

according to methods previously described.

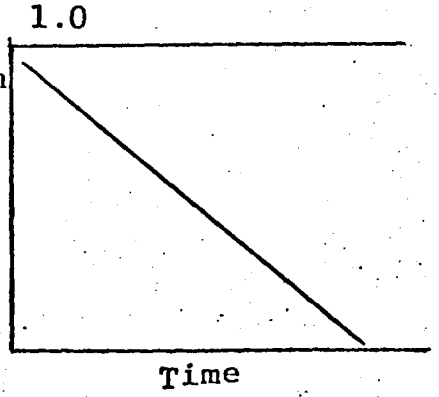
The labeling index of Type II cells and the total number of Type I and II cells in lung will be determined in order to detect any increase in number of cells with age, or a decrease in proliferation of the Type II stem cell with age.

B) Turnover of Type I cells

In this experiment, an attempt will be made to label the precursor cells of Type I epithelium, and follow the persistence of Type I cells by content of radioactivity. Since Type I cells do not normally divide they should maintain their radioactivity throughout their life time. The mean grain count and labeling index of Type I cells will be used to measure their survival.

Labeling of Precursor cells

Epithelium of 17 day mouse lung has a cycle time of approximately 10 hours (8) so that it should be possible to label all epithelium by spaced injections of ^{3}H thymidine over a 24 hour period. The first generations of Type I cells, produced from labeled Type II stem cells will be labeled; and the fraction labeled will decrease at a rate equal to the production of Type I from unlabeled precursors.



The slope of the fraction of labeled Type I cells will thus represent the rate at which new (unlabeled) Type I cells are produced.

1003544049

Source: https://www.industrydocuments.ucsf.edu/docs/nzcb0000

Details of Experimental Design and Procedure

The life span can be estimated by the mean grain count of Type I cells. Since Type I cells are not known to divide under normal conditions, any Type I cell produced from labeled Type II cell should maintain its mean grain count over its lifetime. The mean grain count of Type I cells should not change unless there is turnover of Type I cells. These 2 experiments should answer the questions as to whether alveolar epithelium increases with age and whether Type I epithelium is a renewal or an expanding population.

1003544050

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

BIBLIOGRAPHY

1. Kauffman, S.L. Kinetics of Alveolar Epithelial Hyperplasia in Lungs of Mice Exposed to Urethane. I. Morphometric Analysis of Type 1 and 2 cell populations. Submitted for publication. Lab Invest. 4/73

2. Shimkin, M.B. and Pollisar, M.J. Some quantitative observations on the induction and growth of primary pulmonary tumors in Strain A mice receiving urethane. J. Natl. Cancer Inst. 16: 75-97 1955

3. Frei, Jaroslav V. Toxicity, Tissue Changes, and Tumor Induction in Inbred Swiss Mice by Methyl nitrosamine and -amide compounds. Cancer Res. 30:11-17 1970

4. Walters, M. A. The Induction of Lung Tumors by the Injection of 9-10-Dimethyl 1,2-Benz Anthracene (DMBA) into newborn suckling and young adult mice. Brit. J. Cancer 20: 148-160 1966

5. Kauffman, S.L. , Burri, P.H. and Weibel, E.R. The Post Natal Growth of the Rat Lung. To be submitted. Anat. Rec. 1973

6. Kapanci, Y., Weibel, E.R., Kaplan, H.P. Pathogenesis and reversibility of the Pulmonary lesions of oxygen toxicity in Monkeys. II Ultrastructural and Morphometric studies. Lab. Invest. 20: 101 1969

7. Bowden, D.H. and Adamson, I.Y.R. Reparative Changes Following Pulmonary Cell Injury. Ultrastructional, Cytodynamic and surfactant studies in mice after Oxygen Exposure. Arch. Path. 92: 279 1971

8. Evans, M.J., Cabral, L.J., Stevens, R.J. and Freeman, G. Renewal of Alveolar Epithelium in the Rat Following Exposure to NO_2. Amer. J. Path. 70: 175 1973

9. Kauffman, S.L. Cell cycle times in developing mouse lung - in preparation.

100354051

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

General laboratory, animal rooms, electron microscope laboratory, dark rooms for autoradiography, Wild sampling microscope and morphometry equipment.

11. Additional facilities required: None

100354052

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

13. Publications

1. Kauffman, S.L., Burri, P.H. and Weibel, E.R. Post Natal Growth of the Rat Lung. II Morphometric-Autoradiographic Analysis to be submitted Anat. Rec. 1973

2. Kauffman, S.L. Kinetics of Alveolar Epithelial Hyperplasia in Lungs of Mice Exposed to Urethane. 1. Morphometric Analysis of Type I and 2 Cell populations. submitted for publication. Lab. Invest. 4/73.

3. Kauffman, S.L. Alterations in Cell Proliferation in Mouse Lung Following Urethane Exposure III Effects of Chronic Exposure on Type 2 Alveolar Epithelial Cell. Amer. J. Path. 68:317-325 1972

4. Kauffman, S.L. Alterations in Cell Proliferation in Mouse Lung Following Urethane Exposure. II. Effects of Chronic Exposure on Terminal Bronchiolar Epithelium. Amer. J. Path. 64:531-539 1971

5. Kauffman, S.L. Alterations in Cell Proliferation in Mouse Lung Following Urethane Exposure I The Non-Vacuolated Alveolar Cell. American J. Path. 54: 83-93 1969

1003544053

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

R: REDACTED MATERIAL

BIOGRAPHICAL SKETCH

NAME: Shirley L. Kauffman, M.D.

DATE OF BIRTH: REDACTED

PLACE: REDACTED

EDUCATION:
Northwestern University 1942-44
University of Chicago, B.S. 1946
University of Chicago, M.S. 1948
University of Kansas, M.D. 1955

Hospital Appointments
Provisional Assistant Pathologist, The New York Hospital, July 1, 1955 - June 30, 1957
Trainee of National Cancer Institute assigned to Francis Delafield Hospital, July 1, 1957 - June 30, 1959.
Pathologist, Kings County Hospital 1961 - present
Pathologist, State University Hospital 1966-present
Consultant VA Hospital, Brooklyn 1973

Research and University Appointments
Bacteriologist, U.S. Govt. Food and Drug Administration, 1948 - 1949.
Bacteriologist, University of Chicago, 1950-1951
Assistant in Pathology, Cornell University Medical College, July 1, 1955-June 30,1957
Instructor in Pathology, Albert Einstein Medical College, July 1, 1959-1960
Fellow in Pathology, July, 1960 - December 1960 State University of New York
Assistant Professor, State University of New York January 1, 1961 - 1966
Associate Professor, State University of New York, April 1966 - 1970
Visiting Professor, Dept. of Anatomy, University of Bern, Switzerland, July 1969 - December 1969
Professor of Pathology, State University of New York, September 1970 -

License
American Board of Pathology 1960
License, New York State ; 1957

Society Membership

REDACTED

REDACTED

10035440S4

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s) even if no salary requested)

		% time	Amount
Technicians	I. 10,000		
	2. 8,000		
	18,000		
	.(18)		18,000
			+ 3,240
			21,240

Technical

Sub-Total for A _____

B. Consumable supplies (by major categories)

Fixatives, materials for Epon embedding, slides	800
Glass & Diamond knives and sharpening	1000
Liquid emulsion, isotopes carcinogens	2000
Animals & maintenance	800
	4500

Sub-Total for B 4500

C. Other expenses (itemize)

Photographic film for morphometric analysis	300
Travel to meetings	300
Publication Cost (medical illustration, reprints)	500

Sub-Total for C 800

Running Total of A + B + C 26,540

D. Permanent equipment (itemize)

None

B.K.
R.C.H.

Sub-Total for D 26,540

E. Indirect costs (15% of A+B+C) Overhead 15% of ABC + 3,981

Total request $30,521

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	22,515	4800	800		4217	32,330
Year 3						

10035544055

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Morphometric Study of Mouse Lung Exposed to Carcinogens	The Council for Tobacco Research. U.S.A. Inc.	25,000	Oct.1-Sept.1973 1972

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Pulmonary Carcino-genesis in Fetal and Neonatal Lung	National Institute of Health	$41,000	Oct. 1,1973- Sept. 31,1976

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Acting Chairman

Checks payable to

Mailing address for checks

Principal investigator

Typed Name Shirley L. Kauffman

Signature Shirley L Kauffman Date 7/7/73

Telephone 212 270-1305
Area Code Number Extension

Responsible officer of institution

Typed Name

Title

Signature Date

Telephone
Area Code Number Extension

10035440S6

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000

#572C - LEVY

1003544057

Source: https://www.industrydocuments.ucsf.edu/docs/nzcm0000